

101522074

Fig.1

<u>Pre</u>	<u>Pro</u>	-----pre-albumin----- -----pro region-----
HSA	HSA	Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala Tyr Ser Arg Gly Val Phe Arg Arg
HSA	MFA-1	Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala Tyr Ser Arg Ser Arg Lys Arg
		-24 -23 -22 -21 -20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1
	Preferred mutations of the invention:	Phe Ile Val Ile

Fig.2Standard genetic code

	T	C	A	G
T	TTT Phe (F)	TCT Ser (S)	TAT Tyr (Y)	TGT Cys (C)
	TTC Phe (F)	TCC Ser (S)	TAC Tyr (Y)	TGC Cys (C)
	TTA Leu (L)	TCA Ser (S)	TAA Ter	TGA Ter
	TTG Leu (L)	TCG Ser (S)	TAG Ter	TGG Trp (W)
C	CTT Leu (L)	CCT Pro (P)	CAT His (H)	CGT Arg (R)
	CTC Leu (L)	CCC Pro (P)	CAC His (H)	CGC Arg (R)
	CTA Leu (L)	CCA Pro (P)	CAA Gln (Q)	CGA Arg (R)
	CTG Leu (L)	CCG Pro (P)	CAG Gln (Q)	CGG Arg (R)
A	ATT Ile (I)	ACT Thr (T)	AAT Asn (N)	AGT Ser (S)
	ATC Ile (I)	ACC Thr (T)	AAC Asn (N)	AGC Ser (S)
	ATA Ile (I)	ACA Thr (T)	AAA Lys (K)	AGA Arg (R)
	ATG Met (M)	ACG Thr (T)	AAG Lys (K)	AGG Arg (R)
G	GTT Val (V)	GCT Ala (A)	GAT Asp (D)	GGT Gly (G)
	GTC Val (V)	GCC Ala (A)	GAC Asp (D)	GGC Gly (G)
	GTA Val (V)	GCA Ala (A)	GAA Glu (E)	GGA Gly (G)
	GTG Val (V)	GCG Ala (A)	GAG Glu (E)	GGG Gly (G)

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Single letter code:

A = adenosine

C = cytidine

G = guanosine

10 T = thymidine

B = C or G or T

D = A or G or T

H = A or C or T

K = G or T

15 M = A or C

N = A or C or G or T

R = A or G

S = C or G

V = A or C or G

20 W = A or T

Y = C or T

*Fig.3*Modified list of preferred yeast codons

	T	C	A	G
T	TTC Phe (F) TTG Leu (L)	TCT Ser (S) TCC Ser (S)	TAC Tyr (Y) TAA Ter	TGT Cys (C) TGG Trp (W)
C		CCA Pro (P)	CAT His (H) CAA Gln (Q)	
A	ATT Ile (I) ATC Ile (I) ATG Met (M)	ACT Thr (T) ACC Thr (T)	AAC Asn (N) AAG Lys (K)	AGA Arg (R)
G	GTT Val (V) GTC Val (V)	GCT Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E)	GGT Gly (G)

Fig.4

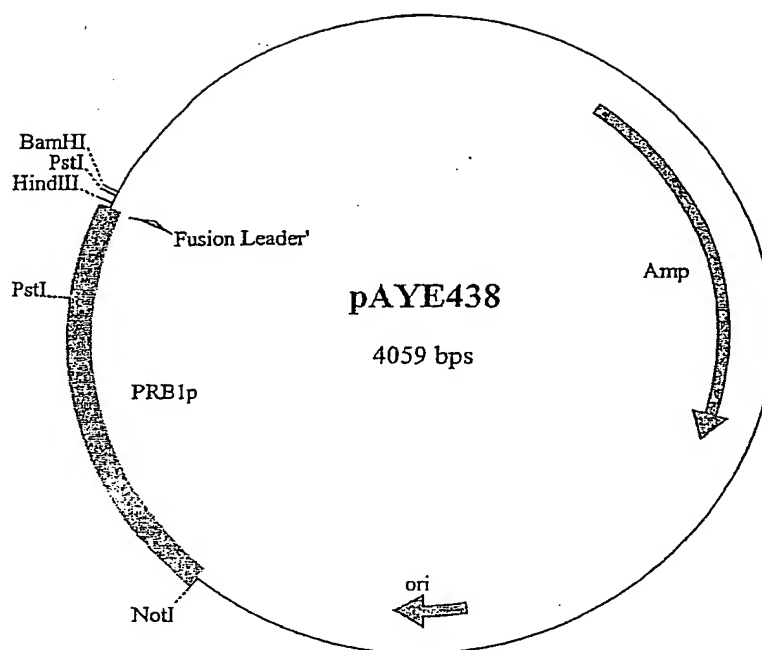


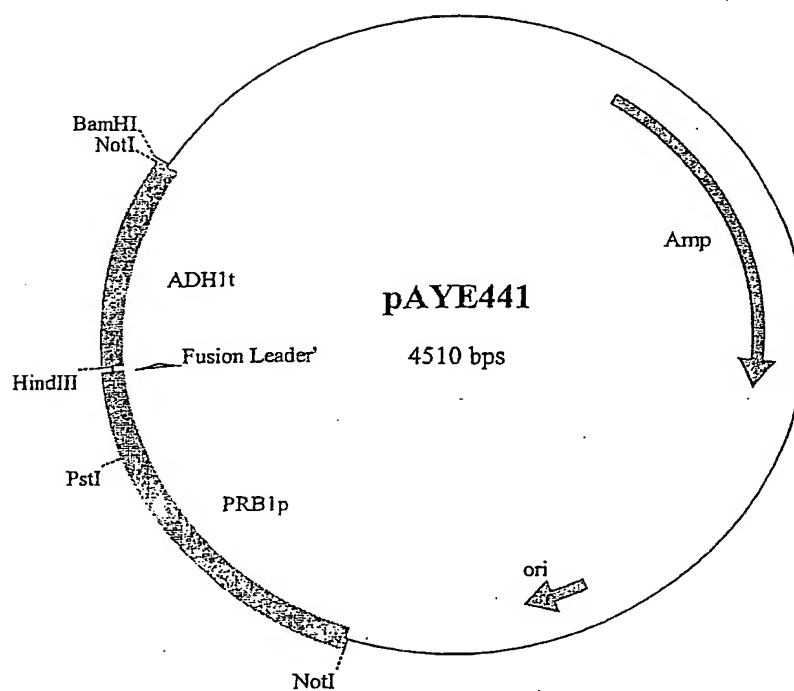
Fig. 5

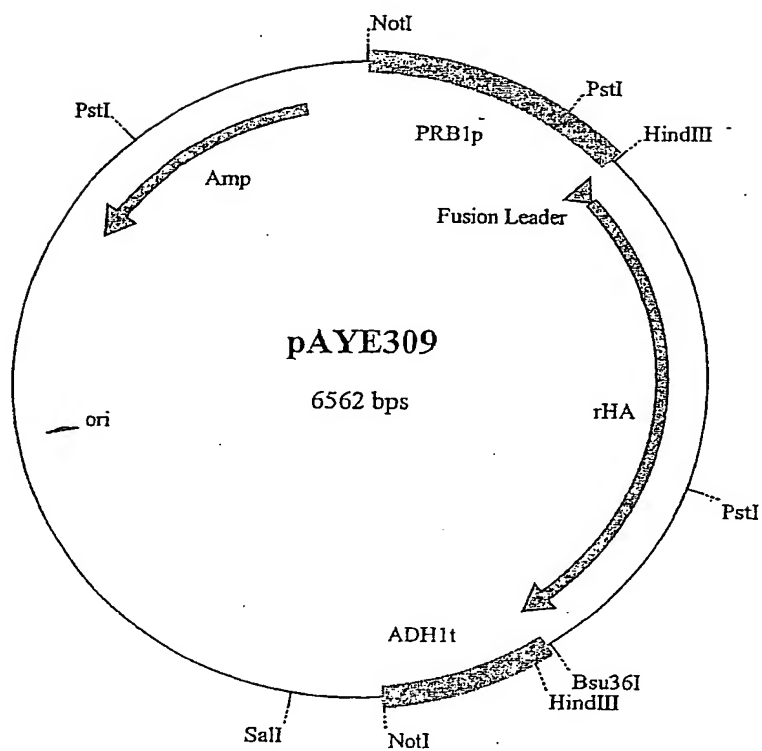
Fig.6

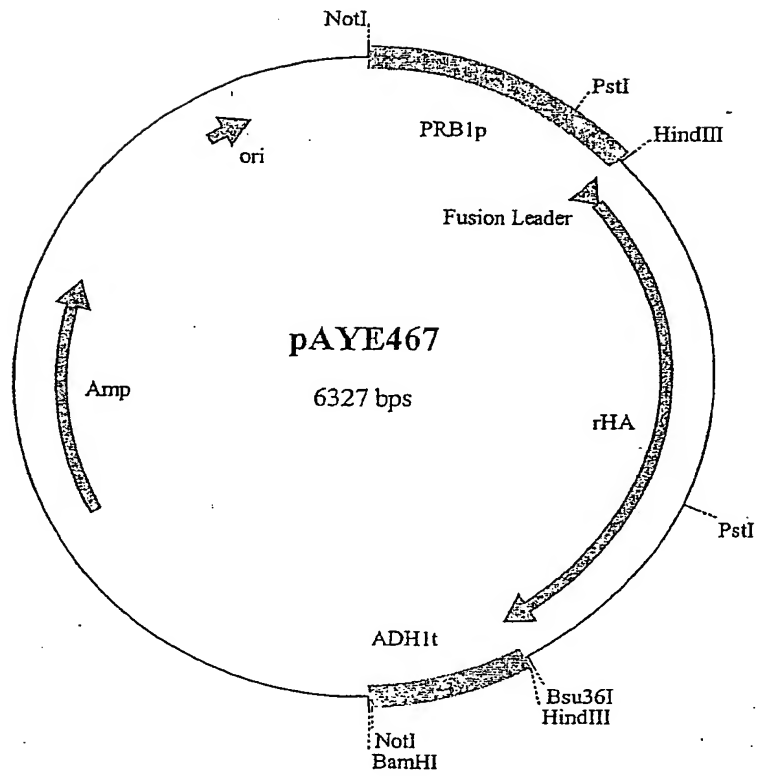
Fig. 7

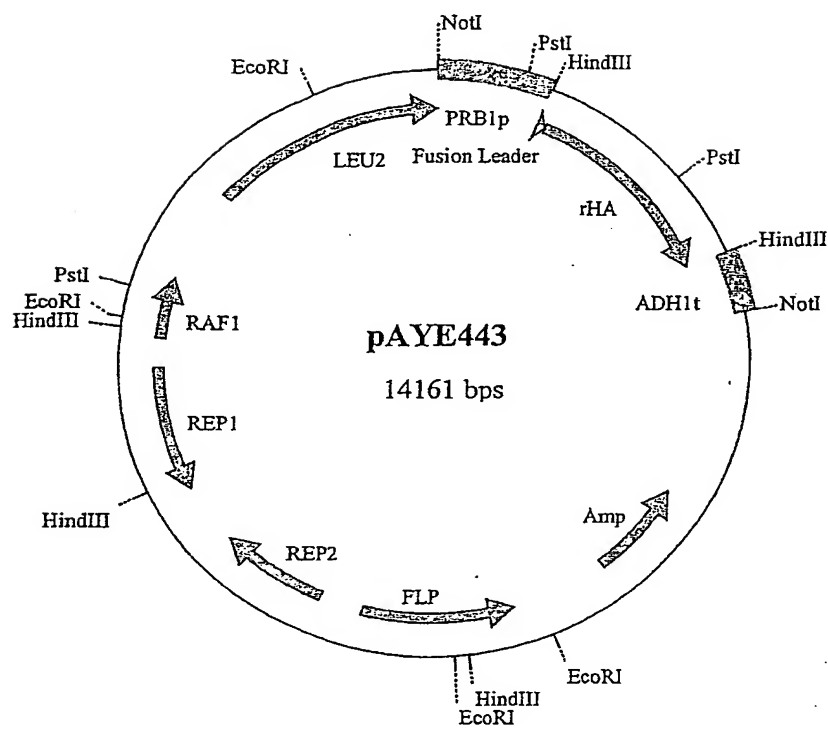
Fig. 8

Fig. 9

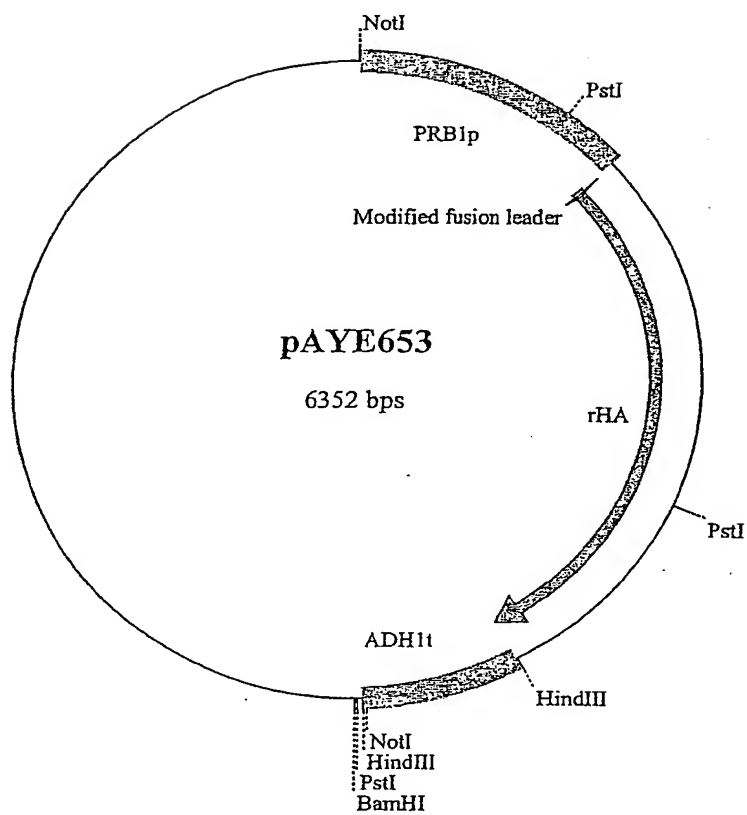


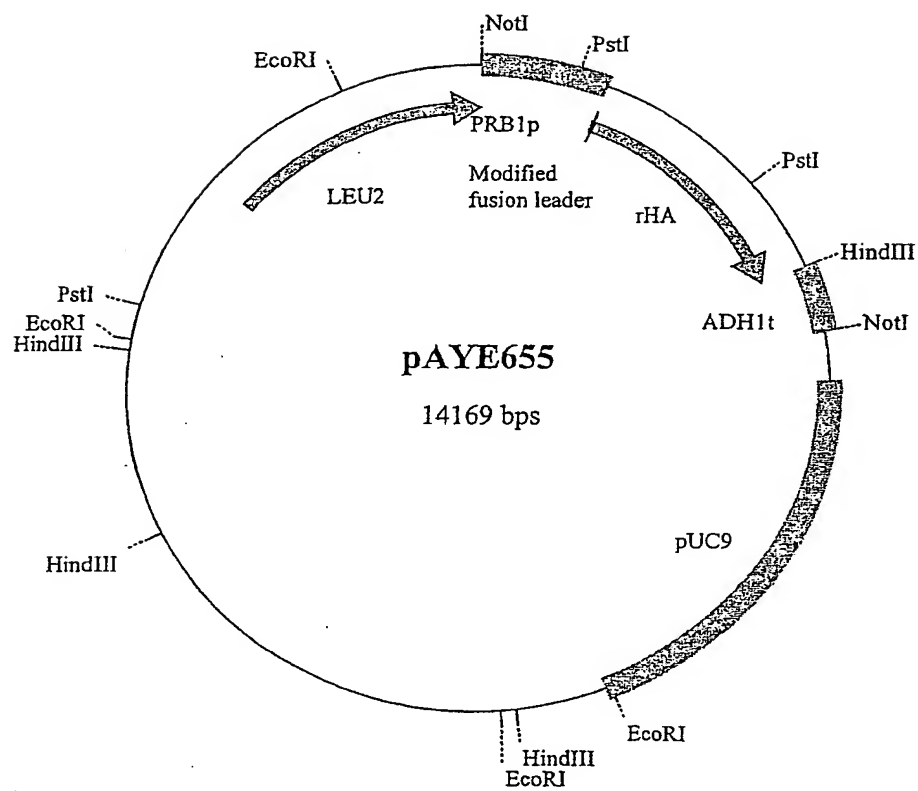
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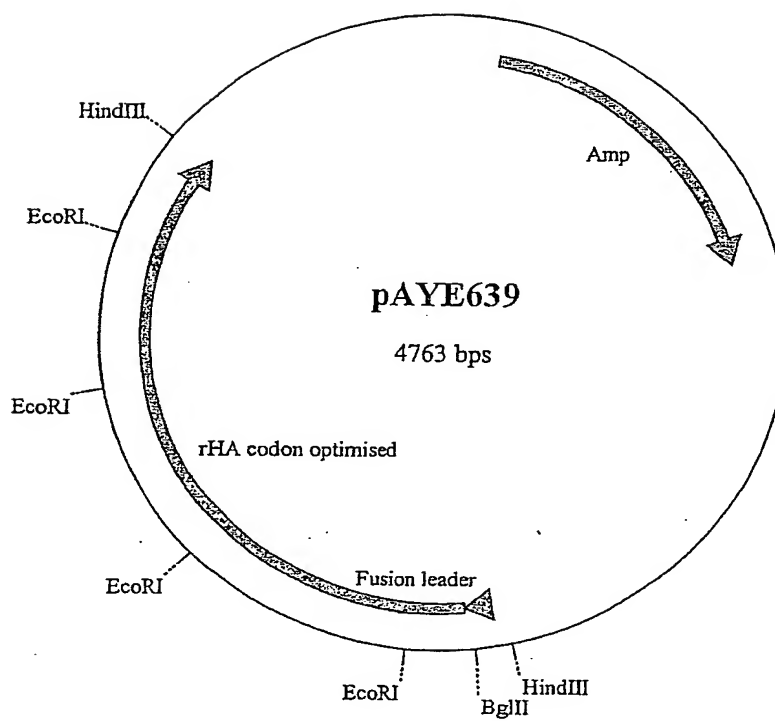
Fig.11

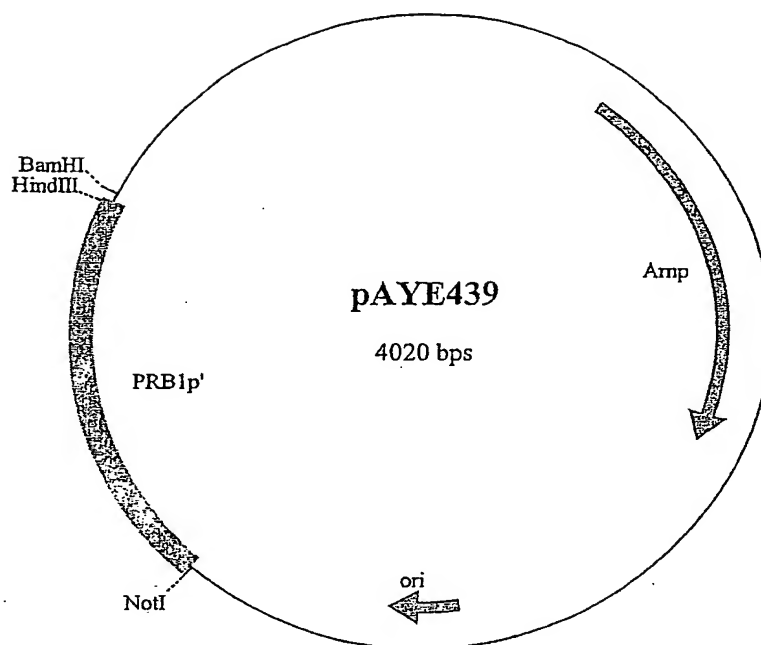
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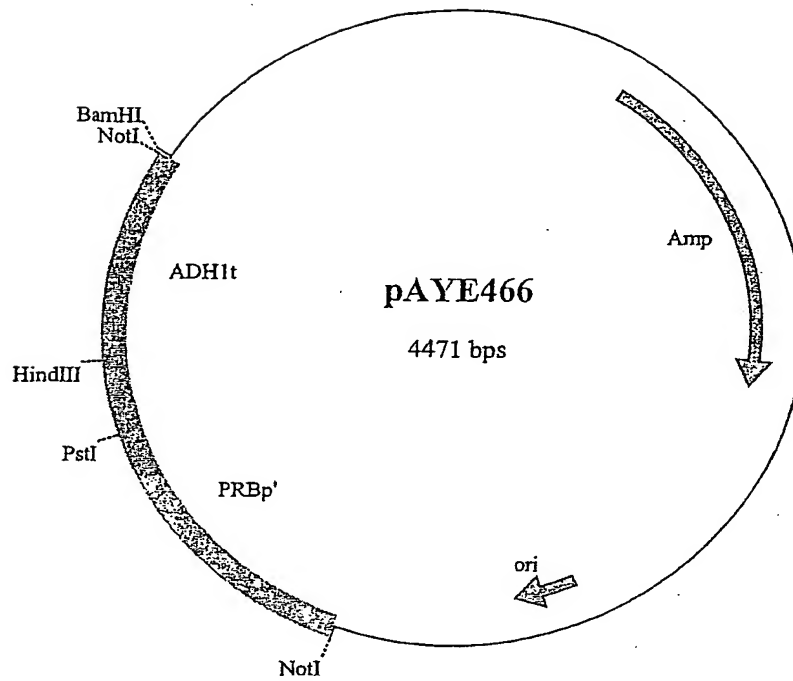
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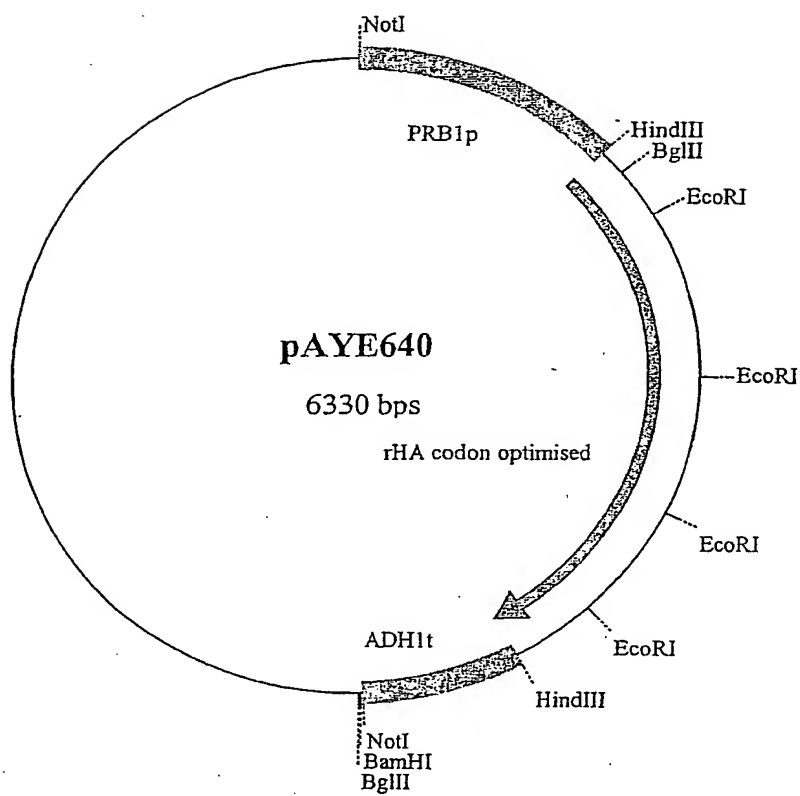
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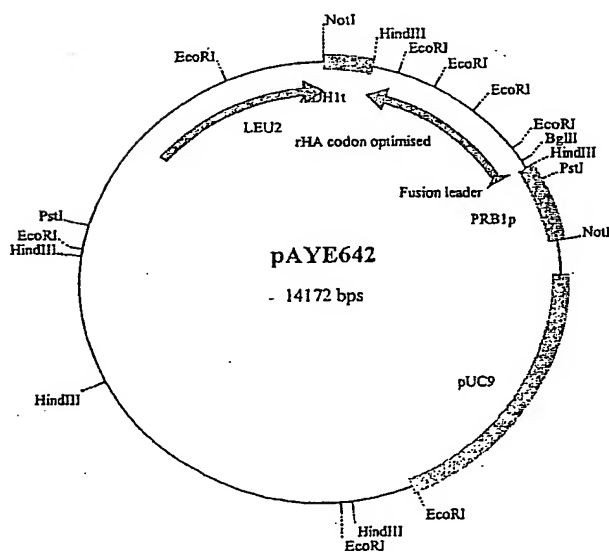
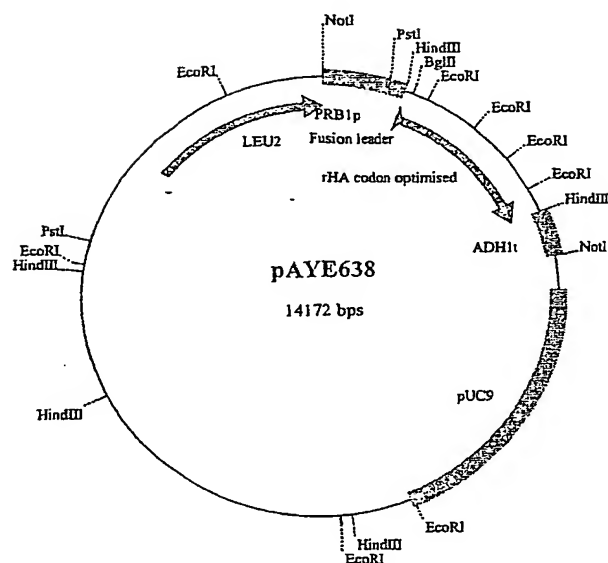
Fig.15

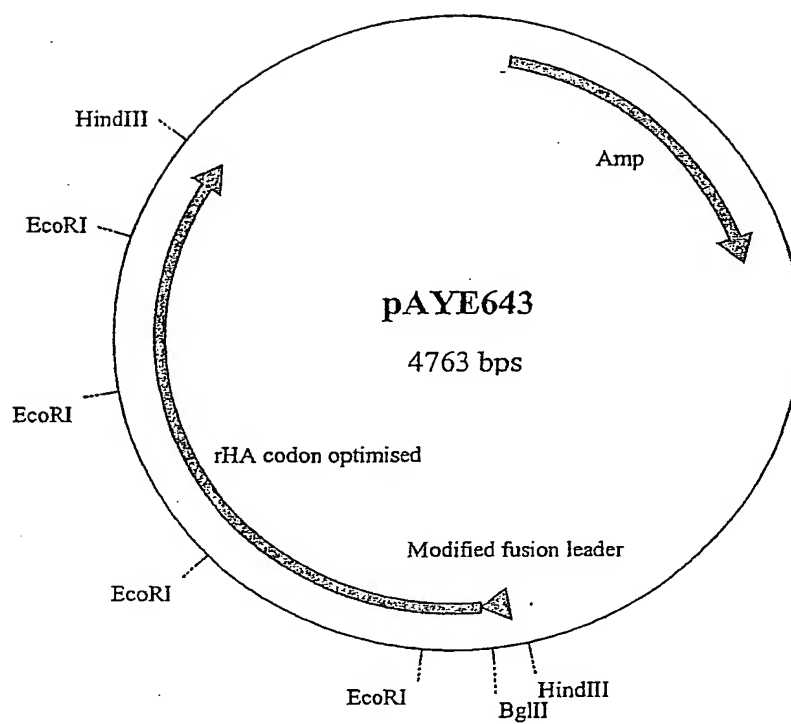
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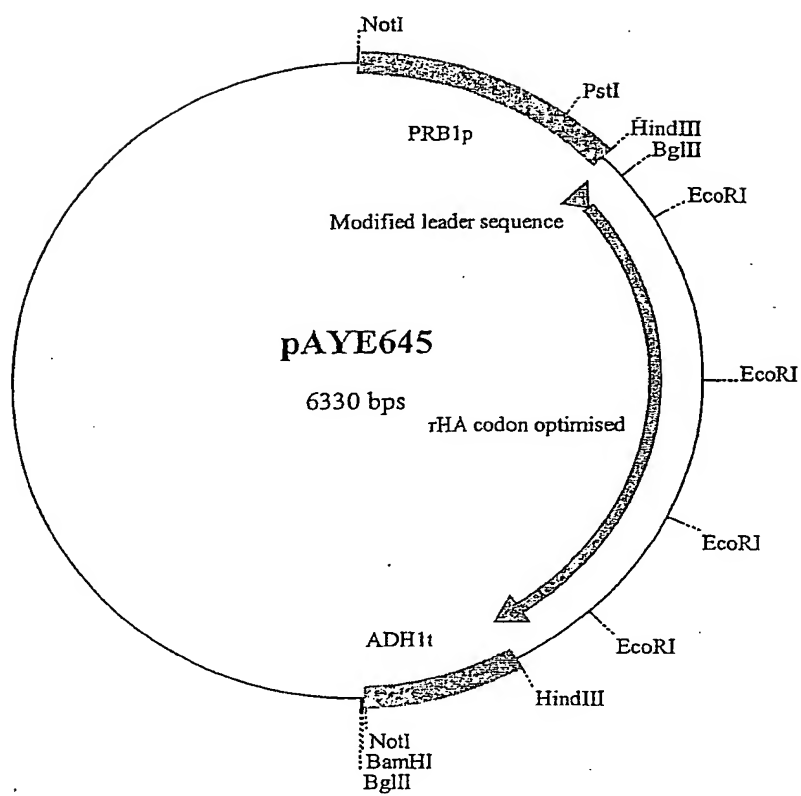
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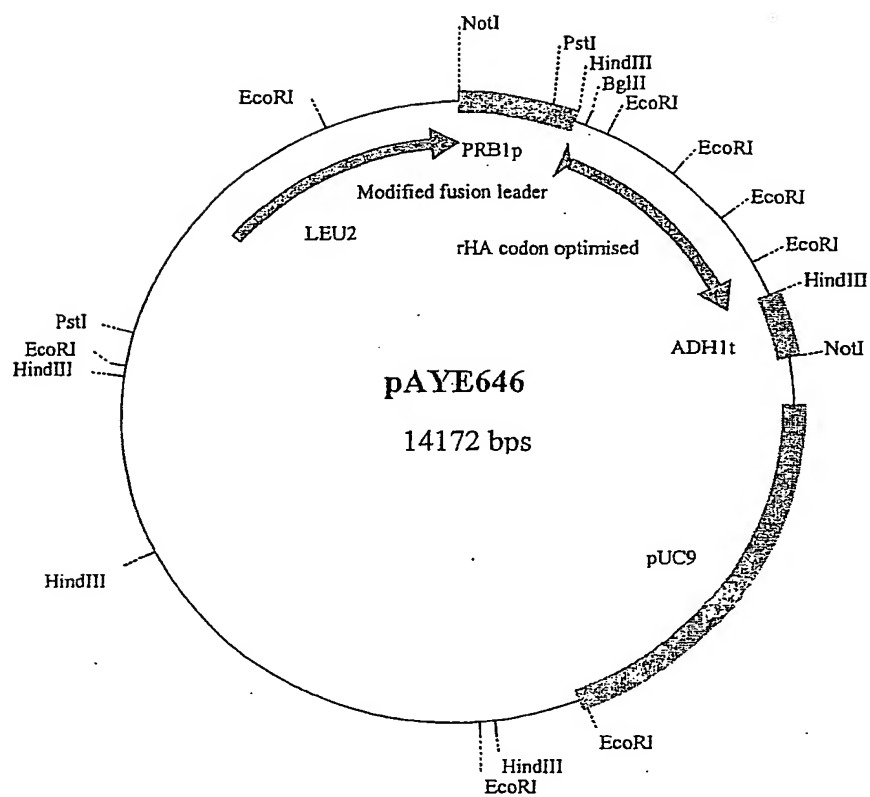
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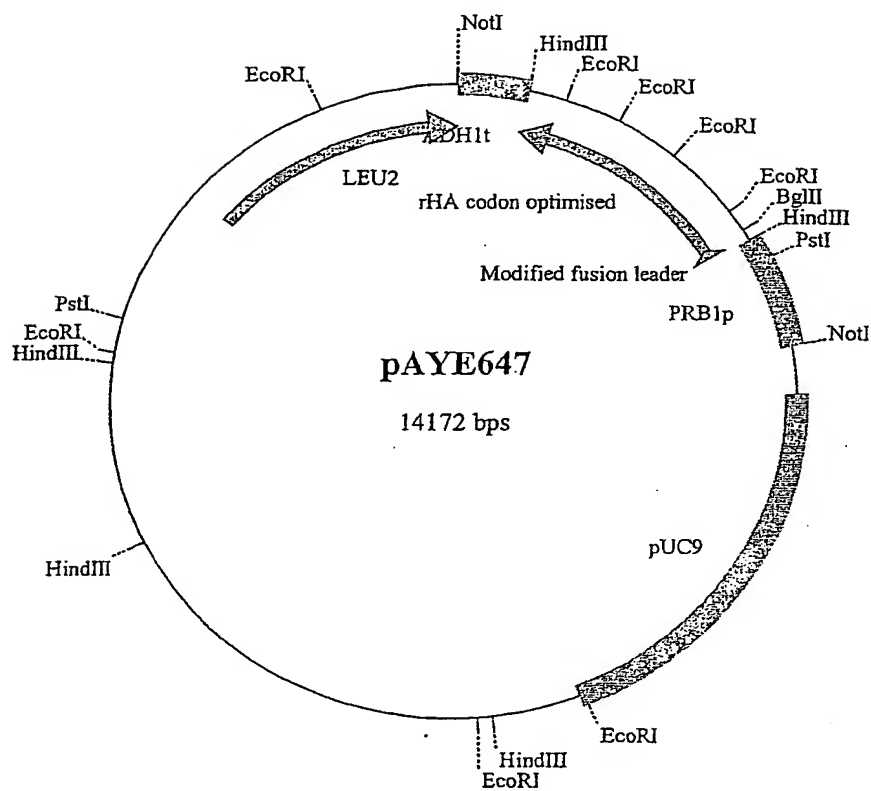
Fig.19

Fig. 20

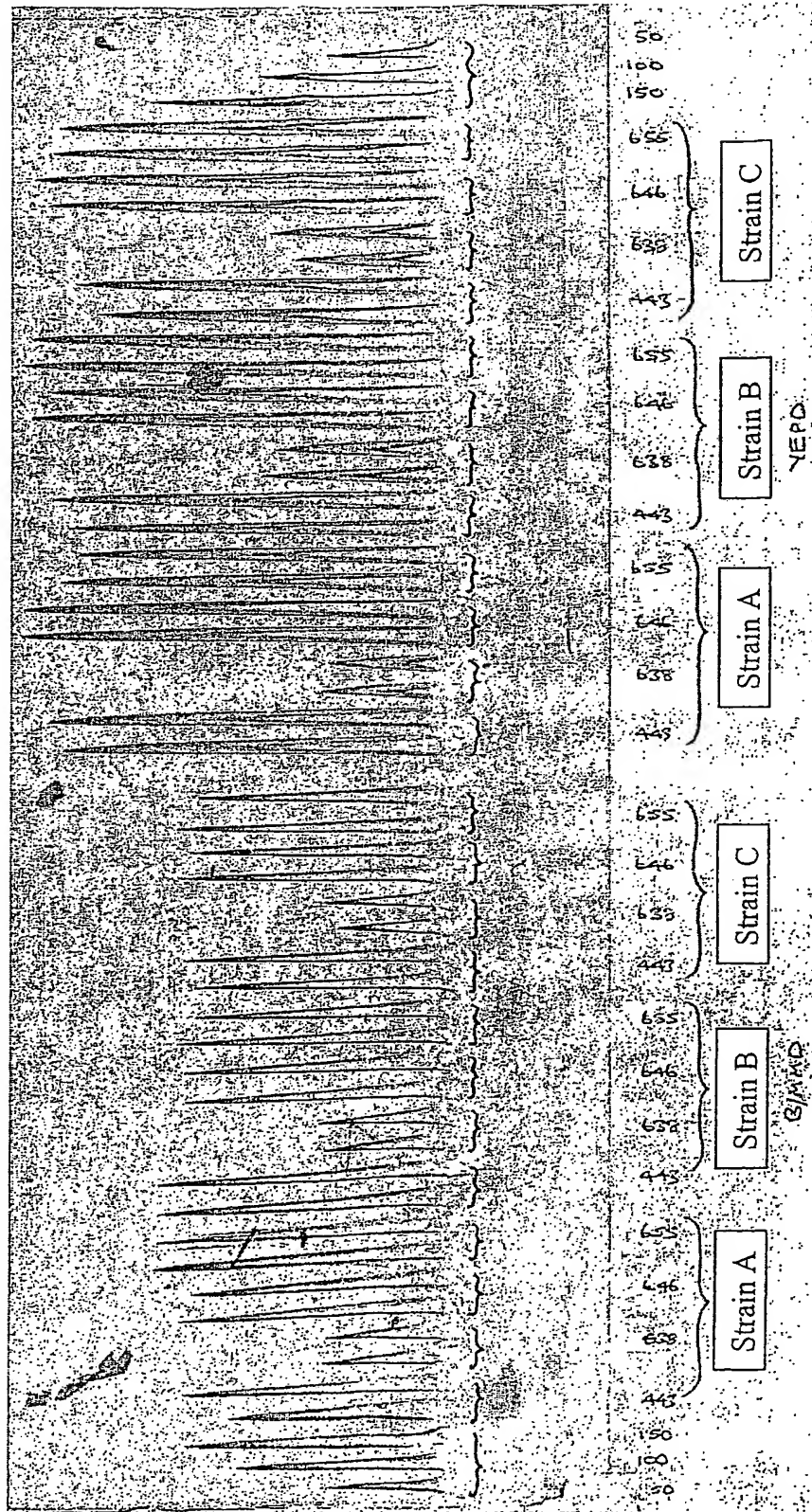


Fig.21

Strain	Plasmid	Yx/s (g/g)	Yp/s (mg/g)	rHA (g/L)
C	pAYE443 (1 st feeds)	0.33	10.4	2.9
	(fill and draw)	0.34	11.5	3.2
	pAYE638 (1 st feeds)	0.36	*	*
	(fill and draw)	0.36	2.4	0.7
	pAYE646 (1 st feeds)	0.33	11.6	3.2
	(fill and draw)	0.35	12.2	3.5
	pAYE655 (1 st feeds)	0.37	12.1	3.4
	(fill and draw)	0.35	13.0	3.7
B	pAYE443 (1 st feeds)	0.35	10.5	2.8
	pAYE646 (1 st feeds)	0.35	13.0	3.5
	(fill and draw)	0.33	12.8	3.6

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Fig.22

Plasmid designation	Leader	Desired Protein
pAYE443 pAYE467	HSA/MF α -1 natural codon bias	HSA natural codon bias
pAYE655 pAYE643	modified HSA/MF α -1 FIVSI fully codon biased the rest has natural codon bias	HSA natural codon bias
pAYE638 pAYE639 pAYE640 pAYE642	HSA/MF α -1 all fully codon biased	HSA fully codon biased
pAYE645 pAYE646 pAYE647	modified HSA/MF α -1 all fully codon biased	HSA fully codon biased

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